Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial

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Summary

Background A recombinant adenovirus type-5 vector-based vaccine expressing the glycoprotein of Ebola Zaire Makona variant showed good safety and immunogenicity in a phase 1 trial of healthy Chinese adults. We aimed to assess the safety and immunogenicity of this vaccine in healthy adults in Sierra Leone and to determine the optimal dose.

Methods We did a single-centre, randomised, double-blind, placebo-controlled, phase 2 clinical trial at Sierra Leone–China Friendship Hospital, Freetown, Sierra Leone. We recruited healthy adults aged 18–50 years who were HIV negative, had no history of Ebola virus infection, and had no previous immunisation with other Ebola vaccine candidates. Participants were sequentially enrolled and randomly assigned (2:1:1), by computer-generated block randomisation (block size of eight), to receive the high-dose vaccine ($1·6\times10^{11}$ viral particles), low-dose vaccine ($8·0\times10^{10}$ viral particles), or placebo (containing only vaccine excipients, with no viral particles). Participants, investigators, and study staff (except two study pharmacists) were masked from treatment allocation. The primary safety outcome was occurrence of solicited adverse reactions within 7 days of vaccination, analysed by intention to treat. The primary immunogenicity outcome was glycoprotein-specific antibody responses at days 14, 28, and 168 after vaccination, analysed in all vaccinated participants who had blood samples drawn for antibody tests. The trial is registered with the Pan African Clinical Trials Registry, number PACTR201509001259869, and is completed.

Findings During Oct 10–28, 2015, 500 participants were enrolled and randomly assigned to receive the high-dose vaccine (n=250), low-dose vaccine (n=125), or placebo (n=125). 132 (53%) participants in the high-dose group, 60 (48%) in the low-dose group, and 54 (43%) in the placebo group reported at least one solicited adverse reaction within 7 days of vaccination. Most adverse reactions were mild and self-limiting. Solicited injection-site adverse reactions were significantly more frequent in vaccine recipients (65 [26%] in high-dose group and 31 [25%] in low-dose group) than in those receiving placebo (17 [14%]; p=0·0169). Glycoprotein-specific antibody responses were detected from day 14 onwards (geometric mean titre 1251·0 [95% CI 976·6–1602·5] in low-dose group and 1728·4 [1459·4–2047·0] in high-dose group) and peaked at day 28 (1471·8 [1151·0–1881·8] and 2043·1 [1762·4–2368·4]), but declined quickly in the following months (223·3 [148·2–336·4] and 254·2 [185·0–349·5] at day 168). Geometric mean titres in the placebo group remained around 6·0–6·8 throughout the study period. Three serious adverse events (malaria, gastroenteritis, and one fatal asthma episode) were reported in the high-dose vaccine group, but none was deemed related to the vaccine.

Interpretation The recombinant adenovirus type-5 vector-based Ebola vaccine was safe and highly immunogenic in healthy Sierra Leonean adults, and $8·0\times10^{10}$ viral particles was the optimal dose.

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Introduction

Ebola virus disease results in mortality as high as 90% in infected human beings and up to 100% in non-human primates, and has become a severe threat to public health worldwide.[1] The 2014 epidemic in west Africa associated with Zaire ebolavirus is the largest outbreak of Ebola virus disease in history, causing around 28 600 cases and 11 298 deaths until October, 2015.[2] Unlike previous outbreaks, this epidemic predominantly occurred in urban areas, affecting both community members and health-care workers.[3,4]

As an emergency response to this epidemic, various vector-based Ebola vaccine candidates have been developed and tested in clinical trials. Several candidate vaccines have shown promising results,[5–8] and a recombinant vesicular stomatitis virus-based vaccine expressing the glycoprotein of Zaire ebolavirus (rVSV-ZEBOV) showed high efficacy in an interim analysis of a phase 3 trial in Guinea.[9] However, more evidence on the safety and efficacy of rVSV-ZEBOV is still needed before its use can be approved.

In a preclinical study,[10] significant protection against Ebola virus challenge was observed in non-human primates

Research in context

Evidence before this study

We searched PubMed for clinical trial reports and ClinicalTrials.gov for unpublished randomised trials, using the search terms "Ebola" or "Ebolavirus" and "vaccine", with no language restrictions, up to Aug 17, 2016. Several clinical trials of Ebola vaccine candidates have been reported, including chimpanzee adenovirus type-3 vector-based Ebola vaccine (ChAd3-EBO-Z), modified vaccinia Ankara vector-based Ebola vaccine (MVA-BN-Filo), adenovirus type-26 vector-based Ebola vaccine (Ad26-ZEBOV), recombinant vesicular stomatitis virus vector-based Zaire ebolavirus vaccine (rVSV-ZEBOV), and adenovirus type-5 vector-based Ebola vaccine. The adenovirus type-5 vector-based Ebola vaccine expressing the glycoprotein of the Ebola Zaire Makona variant has been assessed in a phase 1 clinical trial of 120 healthy Chinese adults: it was safe and immunogenic, and could induce specific antibody and T-cell responses within 28 days of vaccination.

Added value of this study

In this phase 2 trial, we investigated the safety and immunogenicity of this vaccine in healthy Sierra Leonean adults at $8{\cdot}0\times10^{10}$ viral particles or $1{\cdot}6\times10^{11}$ viral particles, and followed up participants for 6 months after injection. This is the first report of this vaccine administered to populations in Ebola-endemic regions (ie, west Africa). Vaccine recipients had high humoral immune responses of glycoprotein-specific antibodies that peaked at day 28 and decreased significantly by about 85% 6 months after injection. Participants receiving $8{\cdot}0\times10^{10}$ or $1{\cdot}6\times10^{11}$ viral particles showed no difference in post-vaccination antibody responses.

Implications of all the available evidence

The adenovirus type-5 vector-based Ebola virus vaccine is safe and immunogenic in Sierra Leonean adults, and the optimal dose is $8{\cdot}0\times10^{10}$ viral particles. However, the short duration of antibody responses raised the need for prime-boost immunisation.

immunised with an adenovirus type-5 vector-based Ebola vaccine, suggesting that this vaccine has potential to be used in human beings. In a phase 1 trial,[11] a recombinant adenovirus type-5 vector-based Ebola vaccine expressing the glycoprotein of the Ebola Zaire Makona variant showed good safety and immunogenicity in healthy Chinese adults after one dose. Thus, we aimed to further investigate the safety and immunogenicity of this vaccine in a larger population from Sierra Leone, which was severely afflicted by the 2014 Ebola virus disease epidemic, and to determine the optimal dose of this vaccine.[12]

Methods

Study design and participants

In this single-centre, randomised, double-blind, placebo-controlled, phase 2 clinical trial at Sierra Leone–China Friendship Hospital, Freetown, Sierra Leone, we recruited healthy participants aged 18–50 years. Participants were eligible if they were HIV negative (confirmed by blood test at enrolment), had no history of Ebola virus infection, and had no previous immunisation with other Ebola vaccine candidates (see appendix for full inclusion and exclusion criteria).

See Online for appendix

This trial was reviewed and approved by the Sierra Leone Ethics and Scientific Review committee and Pharmacy Board of Sierra Leone. We did the study in accordance with the Declaration of Helsinki and Good Clinical Practice. All participants provided written informed consent at least 1 day before eligibility screening. The study protocol is available online.

For the **trial protocol** see http://www.jshealth.com/jgzn/zzjg/ymlcpjs/ymlcpjs_gzdt/201612/W020161214426550507006.pdf

Randomisation and masking

Participants were sequentially enrolled and randomly assigned (2:1:1) to receive high-dose vaccine ($1{\cdot}6\times10^{11}$ viral particles), low-dose vaccine ($8{\cdot}0\times10^{10}$ viral particles), or placebo. Block randomisation (block size of eight) was based on a computer-generated block randomisation list generated with SAS version 9.3 by an independent statistician who had no involvement in the rest of the trial. The vaccines and placebo had identical packaging and were labelled with a randomised code as the unique identifier for each participant. Participants, investigators, and study staff were masked from treatment allocation during the study, except for two study pharmacists who prepared and delivered the study vaccines in ready-to-use syringes to the investigator. The pharmacists had no involvement in any other study procedures and were not allowed to reveal treatment allocation. Staff undertaking laboratory analyses were masked from treatment allocation throughout the study.

Procedures

The study vaccine was developed by Beijing Institute of Biotechnology (Beijing, China) and Tianjin CanSino Biotechnology (Tianjin, China), and contained replication-defective adenovirus type-5 vectors expressing the glycoprotein of Ebola Zaire Makona variant (GenBank number KJ660346). The placebo contained the vaccine excipients only, with no viral particles. We administered double injections of vaccines containing $8{\cdot}0\times10^{10}$ viral particles per dose to participants in the high-dose group (ie, total dose $1{\cdot}6\times10^{11}$ viral particles) and double injections of vaccines containing $4{\cdot}0\times10^{10}$ viral particles per dose to participants in the low-dose group (ie, total dose $8{\cdot}0\times10^{10}$ viral particles), with one injection in each arm. Participants in the control group received two injections of placebo, with one injection in each arm. We observed participants for immediate adverse reactions for 60 min after vaccination and followed them up for solicited injection-site or systemic adverse reactions

occurring within 7 days of vaccination and unsolicited adverse events or medication use within 28 days of vaccination. Serious adverse events were recorded throughout the 6 month follow-up period. HIV tests were done at the end of follow-up for any HIV infection acquired during the study period.

Blood samples were collected from participants immediately before vaccination and at follow-up visits (days 14, 28, and 168 after injection). We assessed Ebola-specific antibody responses against the vaccine-matched glycoprotein with ELISA, reported as 90% effective concentration (EC_{90}; the concentration at which there is a 90% decrease in antigen binding), with a subtraction of the pre-vaccination optical density.[13] ELISA EC_{90} was measured at each timepoint, and optical density was read at 450 nm. A positive antibody response was defined as an ELISA EC_{90} value of at least 10. For ELISA EC_{90} values of less than 10, a value of 5 was used for geometric mean titre calculation. Neutralising antibody titres against human adenovirus type-5 vector were measured with the serum neutralisation assay.[14]

Outcomes

The primary safety outcome was occurrence of solicited adverse reactions (both injection-site and systemic adverse reactions) within 7 days of vaccination. The primary immunogenicity outcome was glycoprotein-specific antibody responses, measured before vaccination and at days 14, 28, and 168 after vaccination.

Secondary safety outcomes were unsolicited adverse events within 28 days of vaccination, serious adverse events during the 6 month follow-up period, and HIV infection rate during follow-up. Severity of adverse events was graded according to the standard guidelines issued by the China Food and Drug Administration.[15] The secondary immunogenicity outcome was titres of neutralising antibodies against human adenovirus type-5 vector.

Statistical analysis

We calculated sample size on the basis of results from a previous phase 1 study,[11] using PASS software (version 11.0). Assuming that 95% of participants respond in the low-dose group, 99·9% respond in the high-dose group, and 5% respond in the placebo group, we used a 2:1:1 randomisation ratio to ensure 80% power at $\alpha=0{\cdot}05$ to show a 10% difference between the low-dose and high-dose groups. We needed at least 207 participants in the high-dose group and 104 each in the low-dose and placebo groups. Assuming a 15% loss to follow-up, we aimed to recruit 500 participants (250 in high-dose group, 125 in low-dose group, and 125 in placebo group). Such a sample size would also produce reliable data for frequent adverse events in each group.

The safety analysis was done by intention to treat, and the primary immunogenicity outcome was analysed in the full-analysis cohort—ie, all participants who were given the vaccine and had blood samples drawn for antibody tests after vaccination. Because immunity to adenovirus type-5 vectors varies among populations and could affect vaccine responses,[14] we did a subgroup analysis of safety and immunogenicity, stratified by baseline adenovirus type-5 neutralising antibody titres (low [≤1:200] *vs* high [>1:200]) across the treatment groups.

Antibody responses were reported as geometric mean titre with 95% CIs. We used χ^2 test or Fisher's exact test for categorical data, ANOVA for log-transformed antibody titres, and Wilcoxon rank-sum test for non-normal data. Statistical tests were done with a two-sided α of $0{\cdot}05$ and analysed by an independent statistician using SAS (version 9.3).

An independent data safety monitoring board (consisting of two public health physicians, one clinician, one epidemiologist, one immunologist, and one biostatistician) was established before the start of the trial to oversee the study process and determine the causal relation between serious adverse events and the vaccine. This trial is registered with the Pan African Clinical Trials Registry, number PACTR201509001259869.

Role of the funding source

The funders of the study were involved in protocol design but had no role in data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 10 and Oct 28, 2015, we recruited and screened 618 healthy adults for eligibility, of whom 500 were randomly assigned to receive high-dose vaccine (n=250), low-dose vaccine (n=125), or placebo (n=125; figure 1). Baseline characteristics were largely similar across the treatment groups (table 1). All participants completed the solicited safety observation period of 7 days. Blood samples were drawn from 496 (99%) participants at day 14, 497 (99%) at day 28, and 493 (99%) at day 168.

132 (53%) of 250 participants in the high-dose group, 60 (48%) of 125 in the low-dose group, and 54 (43%) of 125 in the placebo group reported at least one solicited adverse reaction within 7 days of vaccination (table 2). Most adverse reactions were mild and self-limiting, arising during the first 24 h after injection and lasting less than 48 h. However, in a post-hoc analysis, the occurrence of solicited injection-site adverse reactions differed significantly among the three groups ($p=0{\cdot}0169$). In multiple comparisons based on an adjusted α of $0{\cdot}017$, the difference between the high-dose and low-dose groups was not significant ($p=0{\cdot}9002$), whereas both high-dose and low-dose groups had significantly more solicited injection-site adverse reactions than the placebo group ($p=0{\cdot}0077$ for high dose *vs* placebo and $p=0{\cdot}0361$ for low dose *vs*

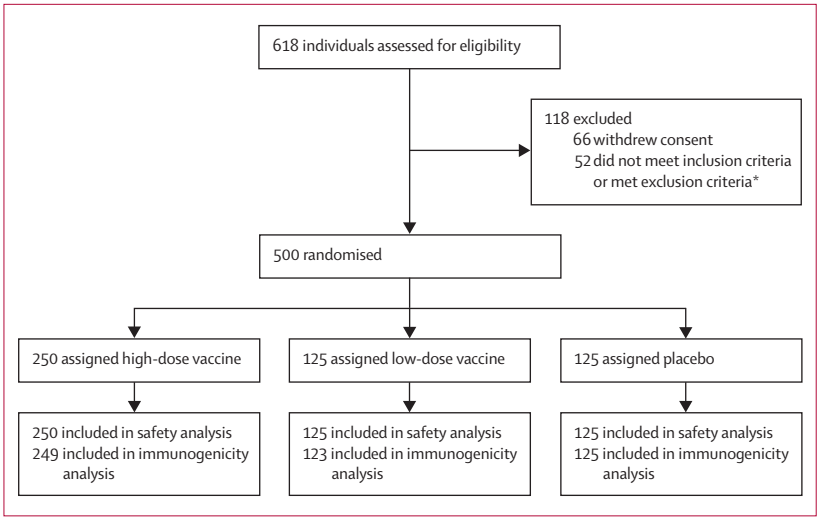

Figure 1: **Trial profile**
*Reasons for exclusion listed in the appendix (p 2).

	Placebo (n=125)	Low-dose vaccine (n=125)	High-dose vaccine (n=250)
Age, years	32·4 (8·4)	32·7 (9·2)	32·2 (8·8)
Sex			
Male	72 (58%)	70 (56%)	126 (50%)
Female	53 (42%)	55 (44%)	124 (50%)
Body-mass index, kg/m²	24·9 (4·2)	24·9 (4·7)	24·7 (4·6)
Pre-existing adenovirus type-5 neutralising antibodies			
Geometric mean titre	109·4 (5·4)	136·7 (4·8)	150·6 (4·4)
>1:12	104 (83%)	105 (84%)	221 (88%)
>1:200	49 (39%)	58 (46%)	117 (47%)

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics**

placebo). The most frequent solicited injection-site adverse reaction was pain, and the most common systemic adverse reactions were headache and fever (table 2). 147 (59%) participants in the high-dose group, 81 (65%) participants in the low-dose group, and 67 (54%) participants in the placebo group reported at least one or more unsolicited adverse reactions within 28 days of vaccination. No particular safety issues associated with pre-existing adenovirus type-5 vector neutralising antibodies were noted (appendix pp 3–5).

During the 6 month follow-up period, one fatal acute asthma episode was reported in a woman aged 44 years who received the high-dose vaccine. She had had an asthma diagnosis for more than 5 years but did not disclose her asthma history at enrolment, since she had not had any asthma attacks for years and had been inhaler free. 5 days after the injection, she reported a moderate cough and difficult breathing to a study investigator, but her symptoms were relieved quickly after taking medications. She felt much better and even participated in an evening church service later that day. However, she had a severe asthma attack around midnight and was declared dead on arrival at the hospital. The data safety monitoring board reviewed all information of this serious adverse event and concluded that it was unrelated to the vaccine before being unmasked from treatment allocation of this participant. Additionally, two participants in the high-dose group reported serious adverse events—one had malaria 4 months after the injection and one had gastroenteritis 5 months after the injection. Both events were considered unrelated to the vaccine and resolved after hospital admission.

Although all participants included in the study had negative HIV screening results at enrolment, six participants (five in the high-dose group and one in the placebo group) were identified as HIV positive at day 168. The occurrences of HIV infection during the follow-up period did not differ significantly among treatment groups (p=0·3223).

Glycoprotein-specific antibody response was detected from day 14 onwards, with at least 96% of responders in both high-dose and low-dose groups at day 14 (table 3). However, the proportion of responders did not differ significantly between the low-dose and high-dose groups at all three timepoints. Glycoprotein-specific antibody response peaked at day 28, with a geometric mean titre of 1471·8 (95% CI 1151·0–1881·8) in the low-dose group and 2043·1 (1762·4–2368·4) in the high-dose group, whereas geometric mean titres in the placebo group remained around 6·0–6·8 throughout the follow-up period (table 3). We noted a transiently higher geometric mean titre in the high-dose group than in the low-dose group at day 28 (p=0·0495), but the differences were not significant at other timepoints (p=0·0671 on day 14 and p=1·000 on day 168). Both the low-dose vaccine and the high-dose vaccine induced strong antibody responses (geometric mean titres >1000) within 28 days of vaccination, irrespective of pre-existing adenovirus type-5 neutralising antibody titres (figure 2). However, the vaccine-elicited antibody responses decreased significantly after the peak: geometric mean titre at day 168 was 223·3 (148·2–336·4) in the low-dose group and 254·2 (185·0–349·5) in the high-dose group. Similar results were also found in the per-protocol cohort (appendix p 6).

Before vaccination, most participants had pre-existing neutralising antibodies against adenovirus type 5, and geometric mean titres were well balanced across the treatment groups at baseline (table 1). Geometric mean titres of these neutralising antibodies increased by more than 10 times in both low-dose and high-dose groups at day 14 (appendix p 7). Although the titres dropped quickly since then, recipients of the low-dose and high-dose vaccines still had antibody titres at day 168 that were 3–5 times higher than that at baseline.

	Placebo (n=125)	Low-dose vaccine (n=125)	High-dose vaccine (n=250)	p value*
Solicited adverse reactions within 7 days				
Any	54 (43%)	60 (48%)	132 (53%)	0·2093
Grade 1	49 (39%)	56 (45%)	121 (48%)	0·2449
Grade 2	10 (8%)	12 (10%)	33 (13%)	0·3039
Grade 3	1 (1%)	0	3 (1%)	0·8110
Solicited injection-site adverse reactions within 7 days				
Any	17 (14%)	31 (25%)	65 (26%)	0·0169
Pain				
Grade 1	14 (11%)	25 (20%)	48 (19%)	0·1009
Grade 2	1 (1%)	1 (1%)	6 (2%)	0·4542
Induration				
Grade 1	0	1 (1%)	3 (1%)	0·8110
Grade 2	0	1 (1%)	2 (1%)	0·8114
Grade 3	0	0	1 (<1%)	1·0000
Redness				
Grade 1	2 (2%)	2 (2%)	4 (2%)	1·0000
Grade 2	0	0	5 (2%)	0·0802
Swelling				
Grade 1	0	2 (2%)	5 (2%)	0·3660
Grade 2	0	1 (1%)	1 (<1%)	1·0000
Grade 3	0	0	1 (<1%)	1·0000
Rash				
Grade 1	1 (1%)	0	0	0·5000
Itch				
Grade 1	2 (2%)	6 (5%)	12 (5%)	0·3138
Grade 2	0	0	1 (<1%)	1·0000
Solicited systemic adverse reactions within 7 days				
Any	45 (36%)	45 (36%)	105 (42%)	0·3954
Fever				
Grade 1	11 (9%)	11 (9%)	20 (8%)	0·9328
Grade 2	2 (2%)	3 (2%)	8 (3%)	0·7158
Headache				
Grade 1	21 (17%)	22 (18%)	56 (22%)	0·3618
Grade 2	6 (5%)	4 (3%)	11 (4%)	0·8778
Grade 3	1 (1%)	0	1 (<1%)	1·0000
Fatigue				
Grade 1	8 (6%)	10 (8%)	22 (9%)	0·7685
Grade 2	0	1 (1%)	3 (1%)	0·8110
Vomiting				
Grade 1	0	2 (2%)	2 (1%)	0·3727
Diarrhoea				
Grade 1	3 (2%)	3 (2%)	3 (1%)	0·5023
Grade 2	1 (1%)	0	1 (<1%)	1·0000
Muscle pain				
Grade 1	4 (3%)	7 (6%)	12 (5%)	0·6696
Grade 2	1 (1%)	1 (1%)	3 (1%)	1·0000
Joint pain				
Grade 1	2 (2%)	7 (6%)	18 (7%)	0·0577
Grade 2	1 (1%)	2 (2%)	1 (<1%)	0·5610
Throat pain				
Grade 1	0	1 (1%)	2 (1%)	0·8114
Grade 2	0	0	1 (<1%)	1·0000
Cough				
Grade 1	1 (1%)	0	4 (2%)	0·4500
Grade 2	0	0	1 (<1%)	1·0000
Unsolicited adverse reactions within 28 days				
Any	67 (54%)	81 (65%)	147 (59%)	0·1938
Grade 1	63 (50%)	78 (62%)	143 (57%)	0·1617
Grade 2	10 (8%)	8 (6%)	14 (6%)	0·6421

Data are n (%), unless indicated otherwise. Grade 1 was mild reaction, grade 2 was moderate reaction, and grade 3 was severe reaction (ie, prevented activity).
*Calculated with χ^2 test or Fisher's exact test.

Table 2: **Solicited and unsolicited adverse reactions**

Discussion

The recombinant adenovirus type-5 vector-based Ebola vaccine was first tested in a phase 1 trial[11] of healthy Chinese adults and had an acceptable safety profile. At a dose of $1·6×10^{11}$ viral particles, the vaccine was highly immunogenic regardless of the presence of pre-existing immunity against the vaccine vector, whereas the immunogenicity of the low-dose vaccine ($4·0×10^{10}$ viral particles) was significantly weakened by pre-existing immunity and the negative effects of pre-existing antibodies against the vaccine vector could not be overcome. On the basis of this finding, we increased the dose from $4·0×10^{10}$ viral particles to $8·0×10^{10}$ viral particles in the low-dose vaccine in this phase 2 trial, and compared it with the high-dose vaccine ($1·6×10^{11}$ viral particles) to further study the safety and immunogenicity of this vaccine in healthy Sierra Leonean adults.

In this phase 2 trial, the high-dose vaccine was associated with increased injection-site reactions, which was consistent with findings of the phase 1 study.[11] However, no severe safety concern of the vaccine was raised, and most adverse reactions were mild or moderate. One participant in the high-dose group had a fatal serious adverse event (asthma episode) 5 days after vaccination. This participant did not report her previous asthma history at enrolment and was therefore randomised and vaccinated. Although this episode was considered unlikely to have been triggered by the vaccine, this individual should not have been included in the study in the first place. We regret that we were unable to identify her history of asthma before she received vaccination.

Results from a preclinical challenge study[10] with non-human primates immunised with adenovirus type-5 vector-based Ebola vaccine showed that a titre of 1000 or

	Day 14			Day 28			Day 168		
	Placebo (n=125)	Low-dose vaccine (n=123)	High-dose vaccine (n=248)	Placebo (n=125)	Low-dose vaccine (n=123)	High-dose vaccine (n=249)	Placebo (n=124)	Low-dose vaccine (n=123)	High-dose vaccine (n=246)
Geometric mean titre (95% CI)*	6·2 (5·2–7·3)	1251·0 (976·6–1602·5)	1728·4 (1459·4–2047·0)	6·8 (5·5–8·3)	1471·8 (1151·0–1881·8)	2043·1 (1762·4–2368·4)	6·0 (5·1–7·0)	223·3 (148·2–336·4)	254·2 (185·0–349·5)
Number of responders (%; 95% CI)*	6 (5%; 2–10)	118 (96%; 91–99)	241 (97%; 94–99)	8 (6%; 3–12)	118 (96%; 91–99)	244 (98%; 95–99)	5 (4%; 1–9)	93 (76%; 67–83)	179 (73%; 67–78)

*Significant differences were noted across the treatment groups, with p<0·0001 at all three timepoints.

Table 3: **Glycoprotein-specific antibody responses**

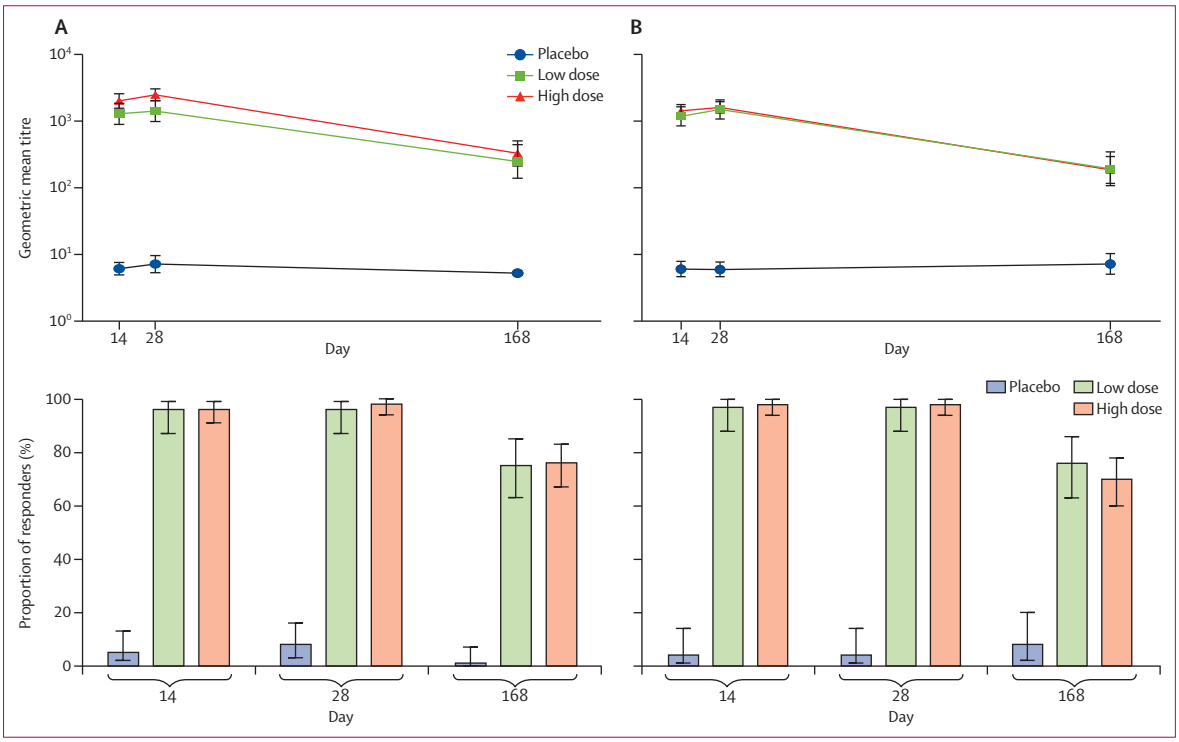

Figure 2: **Glycoprotein-specific antibody responses, by titres of adenovirus type-5 neutralising antibodies at baseline**
(A) Low titre (≤1:200). (B) High titre (>1:200). Error bars indicate 95% CIs.

higher had 77% protection against death. In our study, one shot of the vaccine could elicit strong glycoprotein-specific antibody responses (geometric mean titre >1000) in both low-dose and high-dose groups. Even in participants with pre-existing immunity to the vector, the low-dose vaccine ($8·0×10^{10}$ viral particles) still elicited a humoral response similar to that of the high-dose vaccine ($1·6×10^{11}$ viral particles). Thus, the optimal dose was identified as $8·0×10^{10}$ viral particles. However, durability of the vaccine-elicited specific antibodies was insufficient in the following months, with a much lower antibody titre on day 168 than that observed in Chinese participants who received $1·6×10^{11}$ viral particles in the phase 1 trial (unpublished data). This finding is consistent with other reports of rVSV-ZEBOV vaccine trials in Africa and Europe,[6] suggesting that, in populations from Ebola-endemic regions, protective antibodies are considerably less durable than those in populations from non-endemic regions. This issue deserves in-depth attention, since the populations most in need seem more difficult to protect.

As mentioned in previous reports,[16,17] a concern about the adenovirus type-5 vector is that the activated vector-specific CD4-positive T cells could increase HIV-1 acquisition in vaccine recipients with positive anti-adenovirus type-5 immunity. We did HIV tests at enrolment to exclude HIV-infected individuals from the study. At the end of the study (day 168), five participants in the high-dose group and one in the placebo group were identified as HIV positive, corresponding to annual infection rates of 4% and 1·6 %, respectively. Although HIV infection rates did not differ significantly among the treatment groups, this result is still important to note. Since this finding could be potentially

confounded by false-negative results of participants who were in the early phase of HIV infection at enrolment and 6 months might not be long enough to identify differences in infection risk, an extended follow-up period for new HIV infection is needed to further address this issue.

A limitation of our study was that the adenovirus type-5 vector vaccine platform could be compromised by pre-existing immunity against the vector, since a large proportion of adults worldwide have such immunity.[18,19] For example, more than 85% of healthy Sierra Leonean adults in our study had pre-existing immunity against this vector. We tried to circumvent this problem by increasing the vaccine dose administered. However, our results showed that although a high titre of glycoprotein-specific antibodies could be achieved within 28 days of vaccination at a dose of $8{\cdot}0\times10^{10}$ viral particles or more, humoral immunity was not as robust and long-lasting as we expected. Another limitation was that we did not measure T-cell immune responses elicited by the vaccine because we did not have sufficient laboratory equipment. Although we recruited a relatively large population from an Ebola-endemic region, this single-centre trial might limit generalisability of the results.

Taking vaccine profiles, manufacturing costs, and production capacity into consideration, $8{\cdot}0\times10^{10}$ viral particles seem to be an optimal dose, since it could induce a high level of glycoprotein-specific antibody responses and confer substantial protection to vaccinated individuals, at least in the short term. Thus, the adenovirus type-5 vector-based Ebola vaccine at a dose of $8{\cdot}0\times10^{10}$ viral particles should be investigated in phase 3 trials. However, the short durability of vaccine-elicited antibodies indicates a need for a prime-booster regimen to prolong immunity in future studies. Besides the immunogenicity of this vaccine, its efficacy against Ebola virus disease in epidemic areas still needs to be investigated. Since there is no identifiable high-risk population that can be targeted without the presence of an Ebola epidemic and Ebola outbreaks are unpredictable and sporadic, vaccine efficacy trials after the 2014 epidemic will be very difficult to conduct.

Contributors

AHW was the principal investigator. F-CZ was the co-principal investigator. F-CZ and AHW designed the trial, conducted the trial according to the study protocol, and contributed to critical review and revision of the report. Y-MH, JBWR, QL, and W-JW led and participated in site work, including participant recruitment, follow-up, and data collection. J-XL interpreted the data and drafted the report. L-HH, S-PW, Y-HL, QG, W-BX, ZZ, and W-JY contributed to laboratory analyses, data interpretation, and literature search. MG was the pharmacist of the study. LD and XZ were responsible for the vaccine management. ARW led participant recruitment and follow-up. All authors reviewed and approved the final version of the report. J-ZW and WC supervised the study and had responsibility for all the data.

Declaration of interests

XZ and LD are employees of Tianjin CanSino Biotechnology. All other authors declare no competing interests.

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